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HISTOLOGY OF ASTRAGALUS MOLLISSIMUS. . . . . *Neva Ritter.*

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Histology of *Astragalus mollissimus*.

BY NEVA RITTER.

"LOCO" in Spanish may be translated "crazy." The stock on the range in the western part of America were, therefore, said to be "locoed" when they exhibited symptoms of a disease involving loss of muscular control.

The first published account of the loco-weed disease was in the Report of the Commissioner of Agriculture for October, 1873, by Doctor Vasey. He attributed it to Astragali in California.

From then on every year appeared some new article, more of which were descriptions of the loco weeds and their effects on the stock than reports of discoveries of the true cause of the disease.

Professor Sayre ('86), of the University of Kansas, published articles in the Transactions of the Kansas Academy of Science and in the Report of the State Board of Agriculture describing the loco weeds in Kansas and telling of their effects on stock. He reported two loco weeds here, *Astragalus* and *Oxytropis* (*Aragallus*). He suggested the theory that the hairs on the leaves may by irritation cause the disease. In 1888 he reported having used the extract on himself and getting no resulting loco symptoms. In 1890 he separated some crystals which he said were inorganic and were not the poisonous principle. In 1892 he reported traces of alkaloid and said that loco weeds are not the cause of the disease of the stock. In 1898 he thought a poison develops in the animal which does not exist as such in the plant. In 1904 he put forth three possibilities of the cause of the disease:

(1) Irritation by hairs.
(2) Disturbance to digestion by eating much loco weed in the early spring.
(3) A poison develops during digestion.

Kennedy ('88) found no alkaloid nor poisonous principle when he used infusions in boiling water, dried powder, and an organic

acid from the plant. He experimented on a dog and came to the conclusion that the loco disease is due to the indigestible character of the plant.

Dr. Mary G. Day ('89) made a chemical analysis of loco weed. She experimented on a cat and rabbits. Her conclusions were that there is a poison in loco weed which will cause illness and death, and may be experimentally given and the same results obtained in cats and rabbits; and that the poison must be weak, or if strong, present in a very small amount.

Professor Mayo ('93) gave a good account of the symptoms. He said there is no evidence of a narcotic; the effect is due to malnutrition and malassimilation.

Ruedi ('95) experimented on sheep. In his chemical study he separated "locoin," a base, which he considered harmless, and an acid which is harmful.

Crawford ('08) claimed that barium in loco weeds is the poisonous principle, and tried to support his theory by numerous experiments on sheep and rabbits.

Marsh ('09) told of experiments on feeding and attempts at cure. He said advanced cases are hopeless; they may recover if taken early and put on good feed away from loco weed. He added that injections of sodium cacodylate or strychnine, or the two together for cattle, or Fowler's solution for horses, have been known to help. In 1912 he tried antidotes suggested by Crawford with no success.

Alsberg and Black ('12) tried to substantiate Crawford's contentions for barium, but failed to do so.

The loco disease in Kansas is nearly all due to *Astragalus mollissimus*, the purple loco weed. *Oxytropis* or *Aragallus lamberti*, the white loco or rattle weed, is more plentiful farther west. Of these the stock eat the *Aragallus* more readily, but the *Astragalus* is much the more poisonous. Other species of *Astragalus* and *Aragallus* have been found to be poisonous, but these two only are widespread enough to be of great economic importance.

Astragalus mollissimus of Kansas prairies has a short stem with long pinnately compound leaves, all very hairy, so that they appear silvery. The leaflets vary in number from few to thirty-seven or more. The root goes down quite deep. The flowers are purple, arranged on a spike, blooming the last of May and the first of June. These plants are perennial and remain green all winter. They grow in clumps scattered over the pastures, and are not uniformly distributed. They have many enemies, those

observed last summer being larvæ in the stem, insect galls, fungous diseases, and leaf miners.

Some of the theories proposed to account for the loco disease in stock caused by these plants are:

(1) The weed absorbs the juices of the alimentary tract, causes it to dry up and exert pressure enough to kill the animals.
(2) Overfeeding on loco weeds.
(3) A fungous parasite on loco weeds, Claviceps.
(4) The indigestible character of the plant.
(5) Malnutrition and malassimilation.
(6) Starvation.
(7) Irritation by hairs.
(8) Disturbance to digestion.
(9) Poison developed in digestion.
(10) Not due to loco weeds, but accompanying them.
(11) Alkaloids.
(12) Some poisonous principle not yet isolated.
(13) Barium salts.

The effects of the disease on animals have been described as various. However all agree on a few, as here given by Marsh ('09).

Slow staggering gait.
Rough coat.
Staring look.
Emaciation.
Lack of muscular coördination.
Extreme nervousness, shown in shying, rearing, etc.

To which might be added:

Lack of sensitiveness.
Hallucination.

A horse walking under a telephone wire several feet above its head will lower its head to get under, or when stepping over a stick or a rut in the road will lift its feet very high. The coat of the animal becomes rough, the eyes have a glassy look; there may be, in cattle, a sac of serous fluid under the chin. After the habit of eating loco weeds is formed the animals will hunt for them, and reject other food in preference to them. When locoed they are very hard to manage, and it is almost impossible to back a locoed horse. One cow is reported, when shut up to keep her away from the loco weed after she had the habit, to have run against a stone wall so hard as to kill herself. Starting to walk across a pasture, affected animals keep going until they come against some obstruction. A horse will walk through a barb-wire fence, not

noticing the cuts at all. They finally forget to drink even, and slowly starve to death, hunting loco weeds all the time, and eating nothing else.

If taken from the loco weeds at first they may be cured by shutting them up and feeding, but after the habit has become firmly fixed they are incurable. Prophylaxis is to rid the pasture of loco weeds, but this is practical only in small fenced lots. If fed plenty, so they need no more, in the winter, the habit is not nearly so readily acquired. Loco weeds are usually first eaten in late winter or early spring when the grass is dried up and these plants are still green.

One suggested possibility is that an alkaloid is the cause of the toxicity. Some analyses have shown it, others not. Dean Sayre has found a trace of alkaloid, but as much or more in alfalfa. Miss Watson ('78) found an alkaloidal reaction in the root, also a resinous body. Power and Cambier ('91) found toxic alkaloids, together with volatile oil, acetic acid, resin, albuminoid, and globulin.

Crawford ('08), while working to find the cause of loco poisoning, discovered a trace of barium, which was verified by spectroscopic examination. He measured .01 per cent barium oxide in the ash, which indicated 1.56 milligrams of barium sulphate in 1 gram of ash. In some of the soils in that vicinity he found, by similar examination, no trace of barium, nor in the well water. His experimental results show that barium in the proper amounts, as well as loco weeds, will kill animals; so his conclusion is that barium in loco weeds causes their toxicity. He removed barium with sulphuric acid and found the solution harmless. His extract of the dried material was not always active, which fact he thinks means that there is something in the fresh plant which aids the solution of the barium. His theoretical antidote is sulphuric acid.

Working on this basis, Marsh ('12) experimented with three lots of cattle. One lot were under normal pasture conditions; one was given drinking water containing the amount of sulphuric acid calculated to neutralize the amount of barium eaten in loco weed; the other lot was given drinking water containing a like amount of magnesium sulphate. The same proportion of each lot became locoed, thus showing that neither sulphuric acid nor magnesium sulphate are antidotes, as they should be if barium is the cause of the toxicity, so he makes the statement that barium is not the poison.

A laboratory investigation by Alsberg and Black ('12) on the barium question shows that barium is not responsible for the toxicity of loco weeds. Their evidence is: Many plants which are harmless contain as much or more barium than loco weeds; there are enough other metals present to explain the action attributed to barium; extracts of alfalfa, which are prepared similarly to extracts of loco weed which are toxic to laboratory animals, are found to be toxic to some laboratory animals; the barium is present in "at most but minute traces."

ORIGINAL INVESTIGATION.

ALKALOID. The alkaloid test is a reddish-brown precipitate with bromine or iodine. When the bromine reagent is applied to sections of the petiole, leaflet or stem, and a control (soaked in alcoholic tartaric acid for two days, then in water one day), no difference can be seen in the sections. The same is true when potassium iodide-iodine is used as an indicator. Both of these tests were repeated many times on all parts of the freshly picked plant, indicating no alkaloids.

As a control to the test for alkaloids, sections of nux vomica seeds, calabar beans and belladonna root were soaked in the solvent for alkaloids. These and fresh sections were tested for alkaloids at the same time with potassium iodide-iodine. A very noticeable difference was discernible. The fresh sections show a deep orange-brown color; the alkaloid-free sections a light yellow color, if any. So if an alkaloid were present in my loco weed, a difference in color between fresh and control sections should have been detected; but as there was none, the conclusion reached is that there is no alkaloid present.

SUGAR. Sugar is present in a fairly large amount. When sections of the leaf or stem are boiled in Fehling's solution, at first there is no change, but after boiling longer the brick-red precipitate appears, indicating that cane sugar or a glucoside is probably present. This is indicated in all the cells except the bast fibers and water tubes. Loco weed gathered at 9:30 at night showed the presence of a quantity of sugar in the green parts on boiling with Fehling's solution; that gathered early in the morning not so much.

STARCH. Starch is present in great quantities in the stem, in the pith, medullary rays, pericycle and cortex. In the petiole the starch sheath contains it abundantly, but there is little in the pith.

In the leaflet the palisade cells are full of starch grains. These grains are definite in shape and about three microns in diameter. Potassium iodide-iodine and chloroiodide of zinc are the indicators used.

Material gathered early in the morning showed much starch in the stem, but less in the green parts; that gathered at night showed an abundance in the green parts.

PROTEIN. When Millon's reagent is applied to sections they immediately begin to turn pink, which color deepens on standing or on heating. Protein is thus found to be present in the pith, medullary rays, pericycle, cortex, and especially much in the food-conducting tissues of the stem. In the petiole the same is true except that the pith contains very little. It is uniformly present in the leaflet. Potassium iodide-iodine confirms these indications. Material collected in the early morning and at night seems to contain the same amount.

MUCILAGE. Mucilage is present in the stem, as shown by methylene blue. Sections soaked in water still give the mucilage reaction, showing that it is not completely soluble in water, but might swell. A section of petiole was taken, mounted in alcohol, a distance measured, then water applied at one side of the cover glass. The cells immediately began to swell, the measured portion, sixty microns, swelling to seventy-five microns in a few minutes. The cells could be seen to round out. The mucilage was found to be more abundant near the edge of the section than in farther. The leaflet shows mucilage also. This occurrence of mucilage is interesting, as it is from species of *Astragalus* that the gum tragacanth of commerce is obtained.

OIL. Sudan III shows minute droplets of oil, in the pith cells especially. Sections soaked in ether overnight show less of this. Pieces of stem and leaf were steamed, the stem being collected on cover slips. These were first thoroughly cleaned with ether and handled with forceps. After staining with Sudan III no drops of oil are seen; therefore I conclude no volatile oils are present.

TANNIN. Tannin is indicated by a purple color appearing when ferric chloride is added to sections containing it. No indication of it could be discovered in stem, petiole or leaflet. The test was repeated several times, always on fresh material.

RESIN. Sections were soaked in a saturated aqueous solution of copper acetate, which turns resin green. Observations taken

occasionally for several months show no indications of the presence of resin.

OXIDASES. Gum guiac solution will not turn blue except when oxidases are present. It does not turn blue with any part of loco weed, meaning there are no oxidases present. When hydrogen peroxide is added a dark-red color appears, instead of the deeper blue, which is usual for peroxidases. This may mean a peroxidase is present together with some modifying substance.

GLUCOSIDES. Sections of loco weed treated in the following ways were boiled with Fehling's solution a few minutes, then observations taken:

(1) Fresh sections, no crystals.
(2) Treated with potassium hydroxide overnight at room temperature, cuprous oxide crystals present.
(3) Treated with hydrochloric acid overnight at room temperature, cuprous oxide crystals present.
(4) Treated with sulphuric acid overnight at room temperature, cuprous oxide crystals present.
(5) Treated with alcohol overnight at room temperature, cuprous oxide crystals present.

Untreated sections show crystals on long boiling with Fehling's solution. The same results were obtained when the treated sections were sealed with Fehling's solution and left in a warm place a few days. Sections of material preserved in formalin and treated in the same way give the same results.

These experiments may mean that glucosides are present which are broken by the various reagents used, giving the test by means of the freed sugar.

Crawford says that when sulphuric acid is added to an aqueous solution of harmful loco weed, it is rendered harmless because the barium is taken out of solution and made into barium sulphate. A section of loco weed was sealed with Fehling's solution and kept in a warm place. One week later crystals of cuprous oxide were noticed, showing that no glucose was present at first, or the crystals would have appeared sooner. It was probably a glucoside broken up by long standing and heating in Fehling's solution. Sections were soaked overnight in dilute sulphuric acid at room temperature. The next morning they were sealed with Fehling's solution. After five hours they contained many crystals of cuprous oxide. What happened in a week with Fehling's solution took place in a small fraction of that time with sulphuric acid. I think the change in toxicity in the solution which Crawford used was

due to the glucoside being broken up and thus being rendered harmless, instead of the barium being taken out of solution.

Crawford also states that in dried plants found by him to be inactive the barium has been converted into an insoluble form by drying. Sections of dried plants give a sugar test more readily than formalin or fresh material. This may be due to the glucoside being broken up in the process of drying. Crawford found aqueous extracts of the dried material ineffective, but experiments by others with feeding the dried material show it to be as toxic as fresh. This glucoside is soluble in water, as water in which sections have been soaked for some time show cuprous oxide crystals on heating with Fehling's solution, and the extracted sections have very few crystals in them.

From the experimental data at hand my conclusions are: That the toxicity of loco weeds may be due to some glucoside which is present, but which may be broken apart by various reagents; that the reactions which Crawford ascribed to barium are really due to this glucoside, the presence or absence of which can be proved more readily than the toxicity of such minute quantities of barium as he says are present in the plant.

ANATOMY.

THE LEAF. The leaf is compound, of few to thirty-seven or more leaflets. These as well as the petiole are densely covered by long unbranched hairs. (Figs. 1 and 2.) These occur equally on both sides of the leaf, about fifty per square millimeter. Their occurrence is shown in fig. 3. These hairs are composed of three cells, the basal cell, above this a very short cell with dense protoplasmic contents, and a very long terminal cell with thick cellulose walls. This terminal cell has no cutinized surface, as demonstrated by chloroiodide of zinc, Sudan III, or safranin-hæmatoxylin. There are small irregularities on the surface of this cell of the hair.

These terminal cells of the hair contain no living protoplasm. Methylene blue penetrates the cell wall, but does not color any contents. Hæmatoxylin stains the walls light violet, showing they are cellulose, but stains no contents, the center seeming clear and empty. Potassium iodide-iodine stains the contents of the middle cell deeply, but shows no contents whatsoever in the long terminal cell. Chloroiodide of zinc shows the middle cell stained dark yellow (the wall being cutinized and the contents protein),

but the long terminal cell wall only stains violet, while the cavity looks clear.

Loco weed leaves (dried) absorb 9.24 per cent of water, drops of water disappear when placed on them, and colored solutions (safranin, hæmatoxylin, eosin, and methylene blue) easily penetrate to the cell cavity. Haberlandt says that when such structures are present and the leaf is capable of absorbing much water (by weight) or drops of water when put on them, or there is quick penetration of colored solutions, "it is fairly safe to conclude that the hairs in question represent water-absorbing organs." Therefore I have concluded that these hairs function for the absorption of water in the form of dew or rain. The long cell gathers the moisture, the irregularities on its surface serving to keep the droplets from running off, then the middle cell with its contents conducts it towards the cells of the mesophyll. This middle cell has very dense contents, protein especially. The water may be taken along the terminal cell by imbibition, then drawn by osmosis through the middle cell into the leaf.

Cross sections of the leaflet show little difference in the upper and lower halves. The hairs and stomata (about thirty per square millimeter) are as numerous on one side as on the other. (Figs. 3, 4, and 5.) Palisade cells are arranged on either side of the central portion of the mesophyll, which contains the vascular tissues. It appears that the reflection of the sun from the ground is strong enough to stimulate the formation of palisade tissue on the under side of the leaf as well as to give light enough for the chloroplasts there to function. The terminal tracheids often have enlarged ends, which may serve for water storage. (Figs. 3 and 4.) A section of leaf seen through a microscope with a polarizer attachment shows only the cell walls of the hairs to look bright; nothing else can be seen in the dark field, and no crystals are in this way demonstrated in the leaf. The upper part of the basal cell and all of the outer cell walls of the epidermis are heavily cutinized. (Fig. 4.) A multiple epidermis is shown in a few places. The midvein of the leaflet consists of bast, phloëm and xylem.

The petiole has numerous bundles which supply the leaflets (fig. 6), one going out of the petiole into each petiolule.

There is a layer inside the walls of the bast fibers which stains reddish violet with chloroiodide of zinc, and yellow with potassium iodide-iodine. The lignified walls with both are yellow. With phloroglucin these linings stain red, but a little lighter than the walls. Methylene blue showed no mucilaginous modification.

These layers are therefore concluded to be inner layers of the bast which are not completely lignified, but still have cellulose in them.

With a polarizer, as well as potassium iodide-iodine or chloro-iodide of zinc, starch grains in the starch sheath are shown, but no crystals. Irregular broken spaces are often found in the center of the pith of the petiole. These do not seem to be mucilage channels.

STEM. The stem does not show distinct annular rings, even though secondary thickening is apparent. The phloëm is rather extensive (figs. 8, 9, and 10), and is filled with food materials. Fig. 9 shows three of the smaller bundles in a stem and fig. 10 a longitudinal section of one of the larger bundles. Sieve plates can not be seen. The older portion of the stem has thick cork. This cork tissue gives the suberin test with Sudan III and the lignin test with phloroglucin, as if the suberized membrane were partially infiltrated with lignin.

ROOT. The root shows annular rings better than the stem. Between the rings of water tubes are rings of wood parenchyma cells, which are unlignified, as are also the wood fibers. The water tubes are the only part of the root which is lignified. (Fig. 12.) The phloëm is great in extent. (Figs. 11 and 13.) The older portion of the root has cork similar to that of the stem.

INFLORESCENCE. The peduncle is, like the petiole, made up of many bundles which are to supply the pedicels and bracts at their bases. As these are opposite, the peduncle contains four less bundles above their separation than below. There is relatively more bast in this portion of the plant than in any other. There are one or two rows of lignified parenchymatous cells, making a ring connecting the bundles, near the outer edge of the xylem.

The flower is a typical one of the tribe Galegeæ of the Leguminosæ. The pod is curved, with a partition extending in from the convex side and separating the two cells. In the center of the tissue of the pod is a layer .3 millimeter thick of bast fibers which encircle it, thus making it very stout.

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DESCRIPTION OF PLATES.

PLATE I.

FIG. 1. Hairs taken from a leaf. × 45.

FIG. 2. High-power drawing of hair from leaf, showing: *t*, terminal cell; *m*, middle cell; *b*, basal cell. × 450.

FIG. 3. Surface of a leaflet of loco weed, showing: *t*, tracheal system within the tissue; *e*, epidermal cells; *s*, stoma; *c*, cuticular ring at base of hair; *h*, hair. × 90.

FIG. 4. Cross section of a leaflet at the edge, showing: *e*, epidermis; *b*, basal cell of hair; *h*, hypoderm; *p*, palisade cells; *s*, stoma; *vb*, vascular bundle. × 115.

FIG. 5. Cross section of a leaflet at the midrib. × 115.

PLATE II.

FIG. 6. Diagram of a cross section of the petiole, showing: *e*, epidermis; *b*, bast tissue; *p*, phloëm; *x*, xylem. × 40.

FIG. 7. Cross section of a vascular bundle of the petiole, showing: *e*, epidermis, with *h*, hair; *c*, cortex; *s*, starch sheath; *t*, bast tissue; *p*, phloëm; *x*, xylem; *pi*, pith. × 90.

PLATE III.

FIG. 8. Diagram of a cross section of the stem, showing: *b*, bast tissue; *p*, phloëm; *x*, xylem. × 15.

FIG. 9. Cross section of vascular bundles of the stem, showing: *b*, bast tissue; *p*, phloëm; *x*, xylem; *mr*, medullary ray; *pi*, pith. × 90.

FIG. 10. Longitudinal section of a vascular bundle of the stem, showing the same tissues. × 90.

PLATE IV.

FIG. 11. Diagram of a cross section of the root, showing: *c*, cork; *p*, phloëm; *x*, xylem; *mr*, medullary ray. × 17.

FIG. 12. Cross section of a portion of the xylem of the root, showing: *mr*, medullary ray; *wt*, water tube; *wp*, wood parenchyma; *wf*, wood fibers. × 90.

FIG. 13. Cross section of a portion of the vascular bundle of the root, showing: *p*, phloëm; *mr*, medullary ray; *x*, xylem. × 90.

FIG. 14. Diagram of a cross section of the peduncle, showing: *e*, epidermis; *b*, bast tissue; *p*, phloëm; *x*, xylem. × 30.

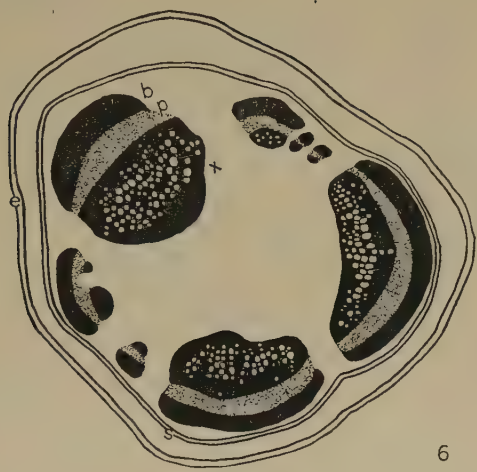
FIG. 15. Cross section of a vascular bundle of the peduncle, showing the same tissues. × 90.

ASTRAGALUS MOLLISSIMUS.
Neva Ritter.

PLATE I.

ASTRAGALUS MOLLISSIMUS.
Neva Ritter.

PLATE II.



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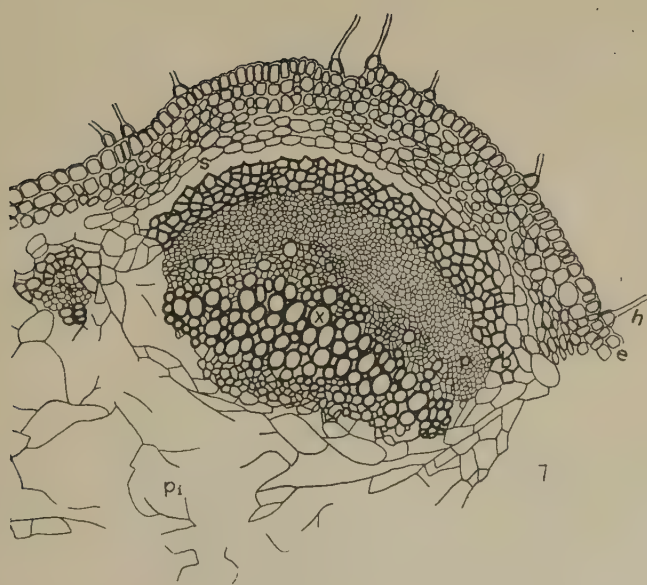

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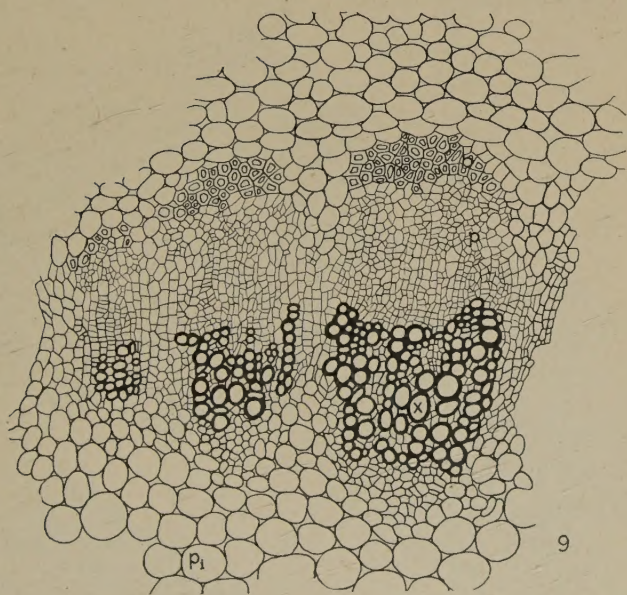
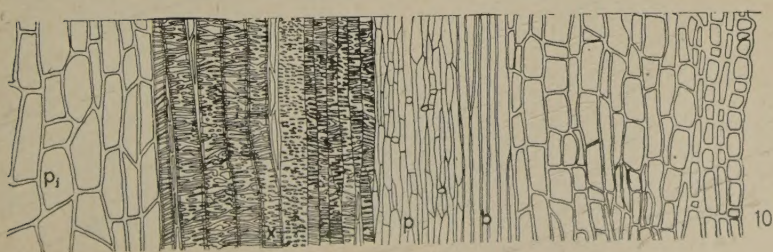
ASTRAGALUS MOLLISSIMUS.
Neva Ritter.

PLATE III.





ASTRAGALUS MOLLISSIMUS.
Neva Ritter.

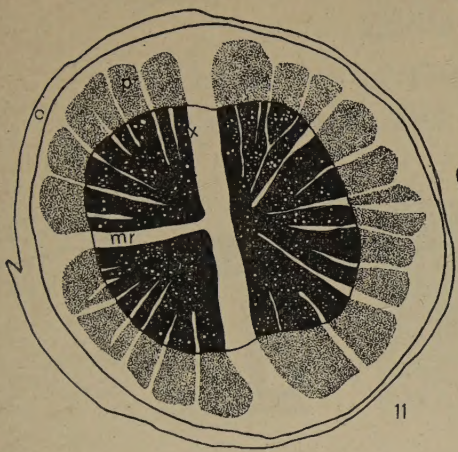
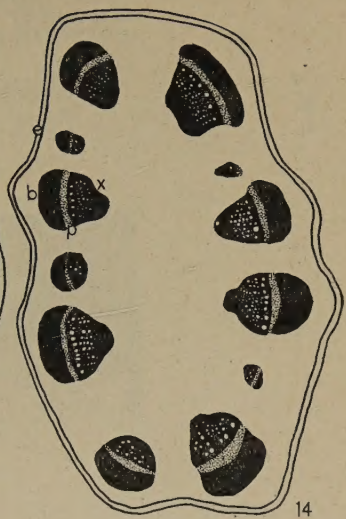
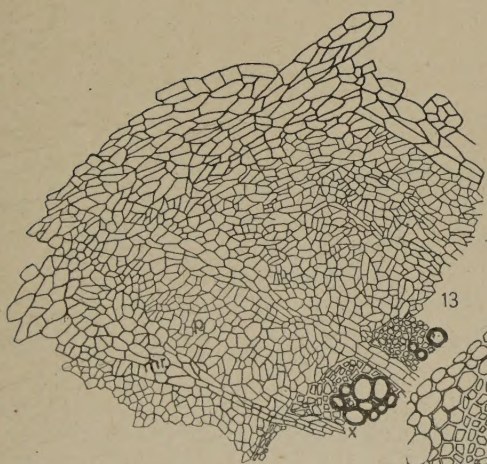
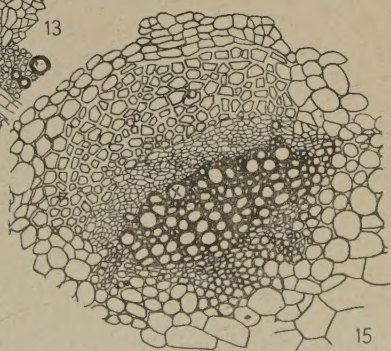
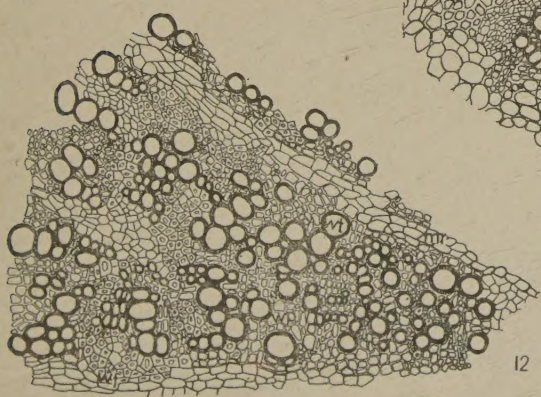



PLATE IV.